

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 April 2004 (08.04.2004)

PCT

(10) International Publication Number
WO 2004/029269 A1

- (51) International Patent Classification⁷: **C12P 17/04**, C12N 9/04
- (21) International Application Number: PCT/EP2003/010495
- (22) International Filing Date: 22 September 2003 (22.09.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 02021624.8 27 September 2002 (27.09.2002) EP
- (71) Applicant (*for all designated States except US*): **DSM IP ASSETS B.V.** [NL/NL]; Het Overloon 1, NL-6411 TE Heerlen (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **HOSHINO, Tatsuo** [JP/JP]; 2-18-14 Fueta, Kamakura-shi, Kanagawa-ken 248-0027 (JP). **MIYAZAKI, Taro** [JP/JP]; Kameino 2-14-8, Fujisawa-shi, Kanagawa-ken 252-0813 (JP). **SUGISAWA, Teruhide** [JP/CH]; Inzlingerstrasse 80, CH-4125 Riehen (CH).
- (74) Agent: **MUELLER, Ingrid**; Roche Vitamins Ltd., Patent Department (VMD), Wurmisweg 576, CH-4303 Kaiser-augst (CH).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- with international search report
 - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
 - with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PROCESS FOR PRODUCING VITAMIN C

(57) **Abstract:** The present invention relates to a process for the production of vitamin C from L sorbosone using an aldehyde dehydrogenase which is isolated from Gluconobacter oxydans DSM 4025 (FERM BP-3812), said enzyme having the following physicochemical properties: (a) molecular weight of $150,000 \pm 6,000$ Da or $230,000 \pm 9,000$ Da (consisting of 2 or 3 homologous subunits, each subunit having a molecular weight of $75,000 \pm 3,000$ Da); (b) substrate specificity as active on aldehyde compounds; (c) Cofactors are pyrroloquinoline quinone and heme c; (d) Optimum pH between 6.4 and 8.2 for vitamin C production from L-sorbosone; and (e) as inhibitors Co^{2+} , Cu^{2+} , Fe^{2+} , Ni^{2+} , Zn^{2+} , monoiodoacetate and ethylenediamine tetraacetic acid. The process is performed in the presence of a suitable electron acceptor and the vitamin C isolated from the reaction mixture.